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TECHNICAL MANUSCRIPT 23

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ABSTRACT

Preliminary experiments on the high frequency (100 and 250 megacycles) internal conductance of Escherichia coli are reported. Observations indicate a difference in conductivity at the two frequencies, which is presumed to be a property of the macromolecules composing the cells. The effect of washing on internal conductivity was investigated by a single set of experiments that indicated definite loss of internal conducting material with washing, the percentage loss per wash decreasing after the third wash, and surprising behavior after seven to nine washes, indicating either a change in cell permeability or osmotic response.

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I. INTRODUCTION

In general, it is difficult or impossible to study directly the properties of the interior of intact living cells. At low frequencies the electrical conductivity of bacteria depends upon the properties of the cell wall. The membrane is essentially "opaque." However, at very high frequencies the reactance of the membrane is so small that it has a negligible effect on the total impedance, and the interior of the cell contributes to the conductivity of the suspension as though the membrane were not present. In this manner, one aspect of the interior of intact cells becomes available for study.

To illustrate possible uses of this technique in the biophysics of bacteria a few preliminary observations of the effect of washing on the internal conductance of Escherichia coli B are reported.

II. EXPERIMENTAL TECHNIQUE

E. coli B grown by the Pilot Plant in nutrient broth were washed, cleaned, and stored at -20°C in cans. Upon thawing the bacteria were washed in distilled water as indicated. A ratio of three or four parts water to one part paste was used for the washings.

Samples for conductance measurements were prepared by adding NaCl to the washed sediment of E. coli. The volume concentration of cells in the slurries was determined from measurements of the dextran-impermeable volume. Thus the conductivities reported are the effective conductivities of the whole cell, including the volume occupied by the cell wall but unaffected by the presence of the membrane.

As mentioned above the membrane controls the effective conductivity of the cell at low frequencies but has a negligible effect at high frequencies. The transition from one condition to the other (the relaxation frequency) for bacteria is in the neighborhood of 10 to 20 megacycles (mc). By 100 or 200 mc the conductance has reached within one to three per cent of the value it would have at infinite frequency. Schwan (1957)* has given the high frequency conductivity as

$$K_\infty = K_a \frac{\varepsilon_\infty}{\varepsilon_a} + p\varepsilon_a\left(2 + \frac{\varepsilon_\infty}{\varepsilon_a}\right)^2 \frac{K_i \varepsilon_a - K_a \varepsilon_i}{(\varepsilon_i + 2\varepsilon_a)^2} \qquad (1)$$

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* Advances in Biological and Medical Physics, Vol. 5, Academic Press, New York.

where K_a and $\mathcal{E}_a$ are the conductivity and dielectric constant of the environment of the cells; $\mathcal{E}_\infty$ is the dielectric constant of the suspension at high frequency; K_i and $\mathcal{E}_i$ are the conductivity and dielectric constant of the interior of the cell; and p is the volume fraction of cells in the suspension. For purposes here $\mathcal{E}_a = 78$. $\mathcal{E}_i$ is estimated to be about 48 on the basis of the solids content and the dielectric decrement of hemoglobin and bacteria given by Schwan (1957). $\mathcal{E}_\infty$ is then calculated from

$$\mathcal{E}_\infty = \mathcal{E}_a \frac{1 + 2p\frac{\mathcal{E}_i - \mathcal{E}_a}{\mathcal{E}_i + 2\mathcal{E}_a}}{1 + p\frac{\mathcal{E}_i - \mathcal{E}_a}{\mathcal{E}_i + 2\mathcal{E}_a}} \tag{2}$$

To calculate K_i, it is then necessary to have a measure of K_∞ and K_a.

To determine these quantities, suspensions of the bacteria were measured in the Boonton R-X meter at 100 and 200 mc. Because of leakage there was a time variation in K_a and K_∞. Simultaneously with the high frequency measurements, the 20-kilocycle conductivity (which is directly proportional to K_a) was monitored in the G. R. Z-Y Bridge. This provided information necessary to correct the observed K_a values to the time at which the corresponding K_∞ was measured. Conversion of bridge readings to sample resistance and capacitance can be made from the following

$$R_x = R_b\left[(1 + \omega^2 L C_b)^2 + \left(\frac{\omega L}{R_b}\right)^2\right] \tag{3}$$

$$C_x = \frac{C_b(1 + \omega^2 L C_b) + L/R_b{}^2}{(1 + \omega^2 L C_b)^2 + \left(\frac{\omega L}{R_b}\right)^2} \tag{4}$$

where R_b is bridge resistance, C_b is bridge resistance less a stray capacitance estimated at 0.15 ρF, and L is the cell inductance estimated to be $1.6 \cdot 10^{-8}$ henries. The cell constant for conductivity was obtained by measuring a 0.05N NaCl solution in the R-X meter and also in the Z-Y Bridge at 20 kilocycles (kc). This calibration procedure tended to take into account variations in temperature in the R-X meter and cell.

III. RESULTS AND DISCUSSION

Figure 1 shows the internal conductance as a function of external conductance for bacteria that had been washed a total of four times before sample preparation. Points include observations from two days. The dispersion indicated by difference between 100- and 250-mc data is presumed to be a property of the macromolecules composing the cells. Hemoglobin shows a similar dispersion (Schwan, 1957). The increase in K_i at high K_a arises in part from increase in conductivity of the cell wall and probably from a real increase in internal conductivity, either because of the osmotic concentration of the internal solutes or by penetration of the salt. At low K_a the internal conductivity exceeds that of the environment. This is possible because of the limitation on swelling provided by the cell wall.

A single series of experiments was performed to investigate the effect of washing on internal conductivity. The conductivity of the bacteria was measured after two, three, five, seven, and nine washes. The results are given in Figure 2. Only the 100-mc data are presented, but dispersion similar to that illustrated in Figure 1 was again observed. There is a definite loss in internal conducting material with washing, but the percentage loss per wash decreases rapidly after the third wash. The surprising observation is in the behavior of the cells at high K_a after seven to nine washes. Either they become somewhat more permeable to the salt or their osmotic response must change. Unfortunately, time prohibits further investigation of this point.

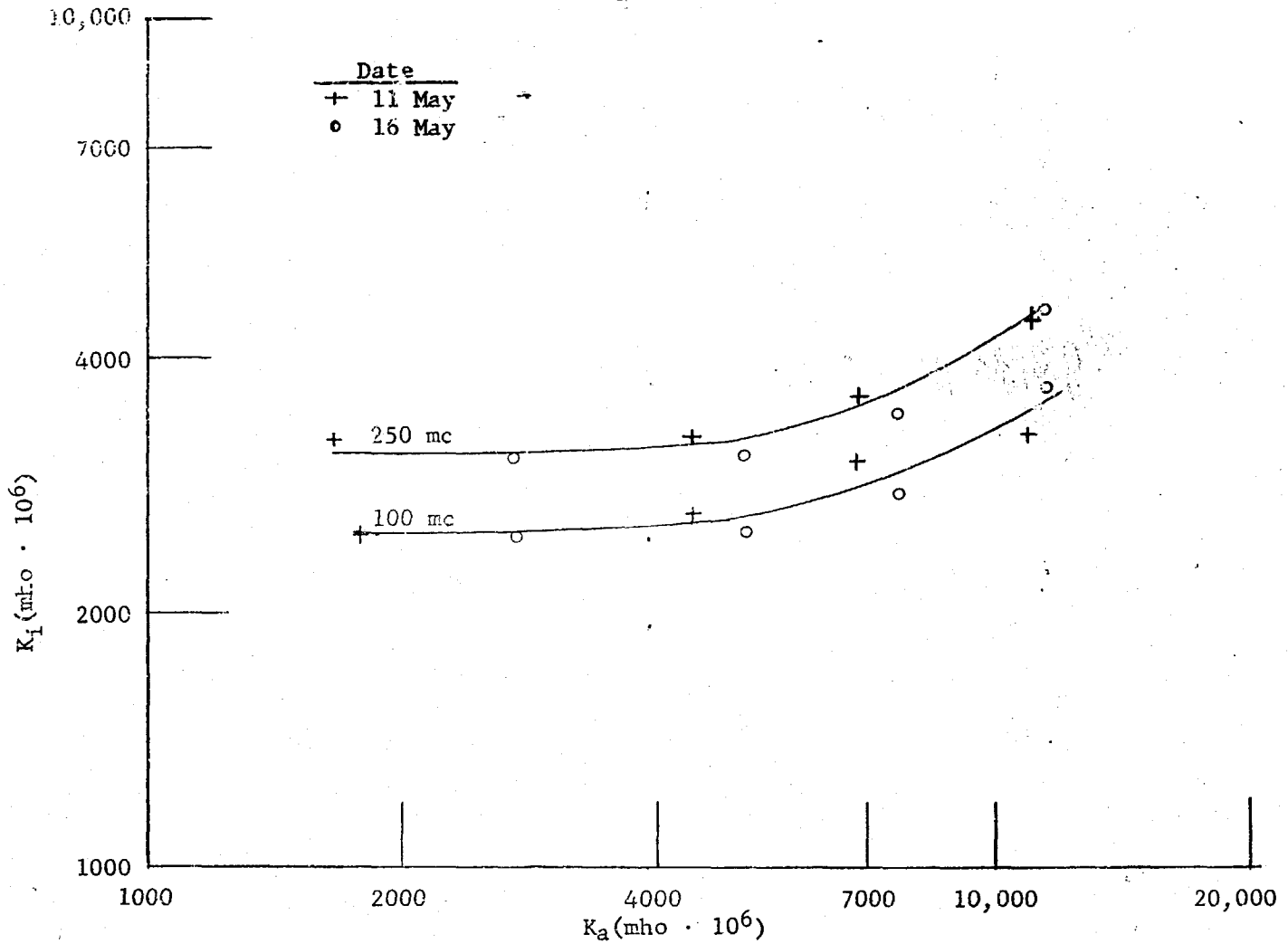

Figure 1. Conductivity K_i of E. coli B as a Function of Conductivity K_a of Environment (measured at 100 and 200 mc). The bacteria had been washed four times before preparation of final slurries.

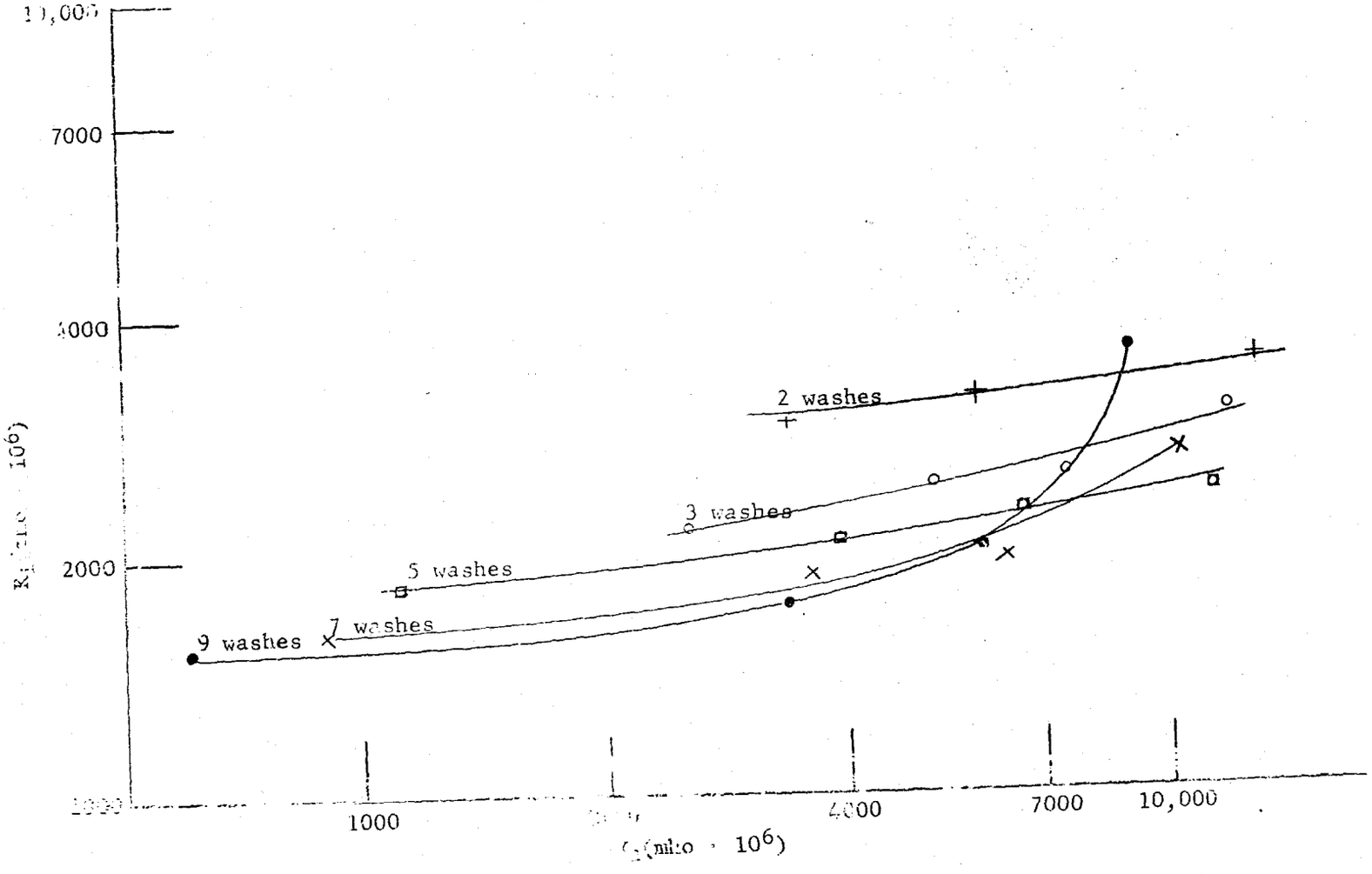

Figure 2. Effect of Washing on Conductivity K_i of *E. coli* B as a Function of Conductivity K_a of Environment. All data are for 100 mc. A similar picture was obtained at 250 mc.